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Second-generation bioethanol production from water hyacinth and duckweed in Izmir: A case study

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ABSTRACT

Water hyacinth and duckweed are both aquatic plants that live in clean water and wastewater. They have excellent filtration ability of nitrogen and supply oxygen to water while cleaning it up. The vegetative reproduction rates of these two plants are really high and it is about 8 days that of duplication time. Because of these features, water hyacinth and duckweed are useful for wastewater treatment. Also production of second-generation bioethanol from these plants is proper due to their cellulose ratios and carbohydrate potentials. In this study, the process of bioethanol production from water hyacinth and duckweed was studied by taking into account the potential of cultivation values and usability in wastewater treatment procedures in Izmir. Different microorganisms, yeast and bacteria (*Saccharomyces cerevisiae, Pichia stipitis*, and *Clostridium thermocellum*) have been considered to identify the best process of ethanol production, considering Turkey's policy regarding biofuels.

Contents

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1. Introduction

Fossil fuels are being used carelessly by humankind and this type of energy cannot supply all of the energy requirements. This has caused CO_2 emissions to increase rapidly, bringing about some changes in the global climate. Hence people begin to search for new alternative/renewable energy sources and technologies/techniques to save the world climate and better the quality of life. New alternative and renewable energy sources, such as solar, wind, geothermal, hydrogen and biomass, are the new sources for supply energy demand for the whole world.

Increasing demand of global energy consumption, mainly used by heavy industries, is the reason for the search of new energy resources. One major economic problem faced by Turkey is importing petrol and its derivatives. To reduce dependence on these valuable sources, bioethanol can be produced because Turkey is a major cereal producer.

Turkey is included in one of the 25 countries that use increasing energy for industrial processes. The Turkish Statistical Institute (TurkStat) published a report named “Greenhouse Gas Inventory” on June 25, 2011, which stated the total greenhouse gas emissions were equal to 369.6 million tons (Mt) of CO_2 in 2009. The biggest part of that emission values comes from the energy sector (75%), followed by wastes and industrial processes (9%).

The importance of using alternative energy sources is increasing globally. Using renewable energies in different sectors, it is possible to decrease greenhouse gas emissions, prevent environmental pollution and increase the domestic production for energy and energy systems.

Instead of petrol, new and renewable energy resources – such as bioethanol – could be considered. Energy requirement is progressively increasing and will keep rising. If the total amount of petrol consumption is taken into account, alternative fuel-oil sources become prominent. This review approaches second-generation bioethanol production from aquatic plants, namely water hyacinth and duckweed, and also the processes of conversion.

2. Situation of Turkey's bioethanol production

Bioethanol, a kind of biomass energy, is an alternative fuel for gasoline. Bioethanol is a clean, safe and renewable resource. It has been considered as a potential alternative to the ever-depleting fossil fuels [1,2].

Traditional production of bioethanol is mainly from sugar, including starchy biomaterials, which is called first-generation bioethanol. However, this causes competition between food and biomass energy sources. Hence, lignocellulosic materials are gradually considered as more attractive renewable resources for ethanol production owing to their easy availability and relatively low cost [3].

In Turkey, production of bioethanol is a new process. First-generation bioethanol production is still carried out by some factories. Table 1 shows the eight factories that produce bioethanol. Most of them are sugar factories that are using molasses to produce bioethanol. Sugar factories only in Amasya, Çumra and Eskişehir perform dehydration and vinasse enriching [4]. Çumra sugar and ethanol factory, TARKIM and TEZKIM are still producing bioethanol and the others have been closed down because of policy and budget problems.

If ethanol production potentials are taken into account, Turkey is a rich and high-capacitive country. Consumption of gasoline for a year in Turkey is approximately 3 million tons (3billion liter). Hence, 1.55 l bioethanol energy corresponds to 1 l gasoline. Related to this situation, 4.65 billion liter of bioethanol is needed to replace bioethanol instead of gasoline. If all bioethanol is produced from wheat, corn, sugar beet or other agricultural materials, which are used as food for human and animals, serious famine and social-economic problems could occur.

Fig. 1 shows the amount of some agricultural products between 2009 and 2010 in Turkey [9,10].

Turkey has good amounts of agricultural materials that can be used for bioethanol production. Merely, their food product values predominate when compared with using with bioethanol production. Hence, second-generation bioethanol has become more important.

3. Using bioethanol as fuel-oil and Turkey's biofuel policy

It is well known that using fossil fuels poses a danger to the environment and health due to its emissions. Gasoline and its alternative fuel ethanol have similar properties (e.g., specific gravity, melting point, and boiling point) and identifications, as shown in Table 2. Comparison of some properties of ethanol and gasoline is given in Table 3.

Ethanol is similar to gasoline, and it is a flammable liquid, requiring a red label by the transportation classification. Its auto-ignition temperature is equivalent to that of gasoline. According to the data on flash point, ethanol is generally considered to be less dangerous than gasoline, since alcohol does not contain light fractions, which means alcohol fire does not start as readily as gasoline fire. It is known that flammable mixtures of air and anhydrous ethanol fuel vapors will be inevitably present in the tank or in the lower part owing to its high vapor pressure and vapor density larger than that of air. The flammability range of ethanol/air mixture at 25 °C is 3.3–19% by volume, indicating that the vapor concentrations in air within that range are explosive.

Table 1
Ethanol factories in Turkey and their production capacities [5–8].

Factory name	Material	Nominal production capacity (l/day)
Erzurum sugar factory[a]	Sugar beet	40,000
Eskişehir sugar factory[a]	Sugar beet	65,000
Turhal sugar factory[a]	Sugar beet	45,000
Malatya sugar factory[a]	Sugar beet	40,000
Çumra sugar factory	Sugar beet	300,000
Tarkim sugar factory	Wheat and corn	100,000
Tezkim sugar factory	Wheat and corn	70,000
Amasya sugar factory	Sugar beet	60,000

[a] Turkey sugar factory member.

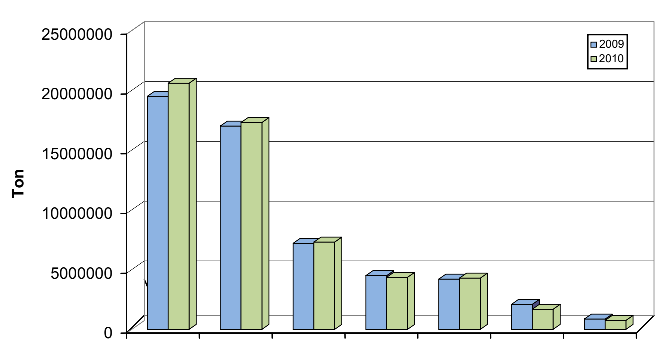

Fig. 1. Amounts of some agricultural products between 2009 and 2010 [10].

Table 2
Some identifications and physicochemical properties of gasoline and ethanol [11].

Identification/physicochemical property	Gasoline	Ethanol
Formula	–	CH_3CH_2OH
Formula weight	~100	46.1
Main synonyms	Benzin, petrol	Ethyl alcohol
Color/appearance	Mobile liquid	Clear, colorless, very mobile liquid
Odor	Characteristic odor	Mild, rather pleasant, like wine
Critical temperature (°C)	–	243.1
Critical pressure (kPa)	–	6383.48
Boiling point (°C)	32–210	78.5
Melting point (°C)	−90.5 to −95.4	−114.1
Specific gravity (as 20 °C)	0.7–0.8	0.789

Table 3
Comparison of fuel qualifications of gasoline and ethanol [11].

Fuel	Vapor density (air=1)	Flash point (°C)	Auto-ignition temp. (25 °C)	Explosive limits (in air, 25 °C)	
				LEL	UEL
Gasoline	3–4	−46 to −36	280–471	1.2–1.4	7.1–7.8
Ethanol	1.6	13 (Closed cup) 18 (Open cup)	363	3.3	19

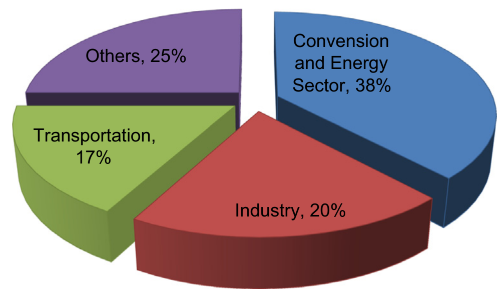

Fig. 2. Turkey's energy consumption according to sectoral allocation [14].

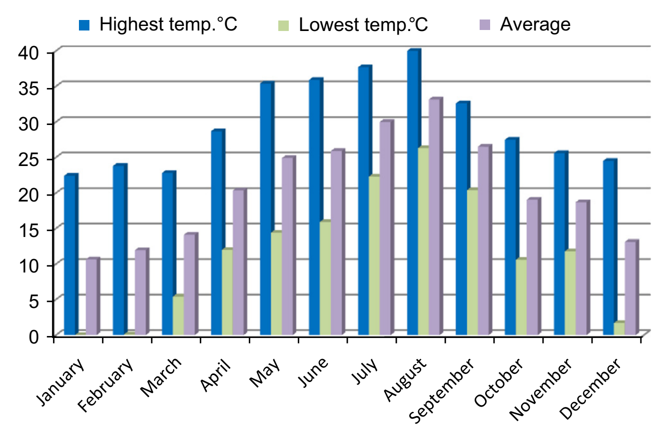

Fig. 3. Temperature changes by months in Izmir.

On the other hand, vapor containing gasoline in air seems safer than ethanol/air mixtures because the flammability range of the former is only 1.2–7.8% by volume [11].

Bioethanol is rarely used in Turkey and some petroleum firms have just started using ethanol as anti-knock. Until 2011, the addition of ethanol to gasoline was decelerated because of legal rules. Although it has been legally allowed to embed up to 5%, only 2% ethanol could be added into gasoline because of the high amount of taxes. Also gasoline prices are really high in Turkey, almost the highest price in the world; hence, using bioethanol should be considered instead of gasoline.

The Republic of Turkey Energy Market Regulatory and Authority (EPDK) has adopted a course of action about using agricultural products as a raw material for biofuel production to promote local biofuel production and to decrease the current deficit. According to this decision from 2013, it is obliged to embed biofuels ever-increasingly from local agricultural products (bioethanol and biodiesel) by the years. Dating from January 1, 2013, the local content rate in gasoline is 2%, and dating from January 1, 2014 it is to be at least 3% [12].

4. Izmir's bioethanol production potential: a case study

Izmir is the 3rd metropolitan city in Turkey and has an expansion of about 12,007 km^2 area. Izmir's total population is 3,965,232 [13]. According to work potential, energy consumption is very high in Turkey's share. Fig. 2 shows Turkey's sectoral energy consumption percent for each industry.

Izmir is in located in the temperate climate zone; winters are not very cold and not snowy, and summers are mostly clear and sunny. The temperature changes by months in a year are given in Fig. 3.

Izmir's agriculture has rich variations. Different kinds of products can be produced in Izmir because of the soil quality and air conditions. A great part of the population is engaged with agriculture and it is really important to harvest efficient product by the seasons. Table 4 shows the amounts of some agricultural products and their usable residues for Izmir. Most of the residues are used for animal feeds. Villagers consider that using residues as feed is better than using them as raw material for any other processes. They do not thus have to pay for feeding materials, which turns out cheaper than buying.

Table 4
Some agricultural products and their usable residues in Izmir for the year 2010 [15,16].

	Production (ton/year)	Residue potential (ton/year)	Usable residue amounts (ton/year)
Barley	31,840	34,270	5141
Wheat	208,224	217,419	32,613
Oat	8960	8916	1337
Corn	1,941,449	4,367,080	2,620,248
TOTAL	2,190,473	4,627,685	2,659,339

Values of cereals and other agricultural plants have prompted researchers to find other sources that do not have any value and is

Fig. 4. Wetlands in Izmir: (a) Gediz Delta; (b) Gebekirse Lake; (c) Belevi Lake; and (d) Golcuk Lake.

not usable for anyone. So, lignocellulosic materials have become prominent. Especially, aquatic plants, which have a rapid growth rate and have an ability to clean wastewater, can be considered. Water hyacinth and duckweed are some of the aquatic plants that can be used in bioethanol production, and Izmir suitable geographic location allows the production of these plants.

Izmir's air conditions make it conducive to grow water hyacinth and duckweed in the wetlands. Izmir has 4 wetlands: Gediz Delta Wetland, Gebekirse Lake Wetland, Belevi Lake Wetland and Gölcük Lake Wetland [17]. The views of these four wetlands are given in Fig. 4.

4.1. Natural wetlands in Izmir

Natural wetlands implicate various plants and aquatic animals. These wetlands help in maintaining the environmental balance with all living species. Most of the plants that sprout in the wetlands also can clean the water. These plants are *Phragmites austrialis* (common reed), *Typha latifolia L.* (broad-leaf bulrush), *Juncus acutus L.* (sharp, pointed reedmace), *Lemna minor L.* (duckweed) and *Eichhornia crassipes L.* (water hyacinth).

According to international criteria, 8 situations should be fulfilled in the wetlands. There are 135 wetlands which observed with criteria in Turkey and it is considered that the number will go up to 200 after the improvement works. There are 300 wetlands of national concern, in Turkey, covering about 3 million hectare.

All wetlands are supplied by underground and terrestrial water, so the most important threat for wetland is water pollution. Aquatic systems have a capital importance to balance the water, in preventing pollutants from reaching potable water and removing and treating wastewater. There are 4 important wetlands in Izmir: Gediz Delta, Gebekirse Lake, Belevi Lake and Gölcük Lake.

Gediz Delta is one of the most important wetlands in Turkey and also in the Mediterranean region. This wetland's size is 40,000 ha and involves fishgarths, alluvial soils, fresh and salty water marshes, seasonal hydrophore fields, Mediterranean-type heath family, agricultural fields and different biotopes. In winter, Gediz Delta Wetland becomes host to 80,000 aquatic birds, of which 28 are endangered species as per Europe's criteria. 700 plant spices have been detected, and 11 of them grow only in the Gediz Delta Wetland. 62 different types of planktons can live in the delta. Investigations on the flora of the delta have revealed *Salicornia europaea* (glasswort) to be the most endemic plant, followed by *Phragmites australis* (nutgrass) and *Lemna minor* (duckweed) [18].

Gebekirse Lake is located in Zeytinkoy/Selcuk, Kucuk Menderes Delta, spanning an area of 839.2 ha. Both freshwater and salty water aquatic systems are found in the lake. This area has been pronounced as a wildlife protection area [19].

Belevi lake is located in Selcuk, Kucuk Menderes Delta. Its size is 150 ha and hosts various bird species. Belevi Lake is a freshwater lake and very suitable for the production of aquatic plants [19].

Golcuk lake was pronounced as a wetland in 2006. It comprises up to 80 ha. It hosts different kinds of birds and plants. The kind of water is fresh water and located in Torbali [19].

4.2. Wastewater treatment facilities in Izmir

Izmir is a metropolitan city and its industry is increasing continuously. Water usage, also wastewater amounts are increasing depending on sectorial applications. There are high levels of industrial wastewater and domestic wastewater in the city; thus, treatment facilities are urgently needed.

In Izmir, 24 wastewater treatment facilities have been built. Most of them are technologic and use chemicals, biological populations and special equipment. 5 of them are natural wastewater treatment plants. The facilities are shown in Table 5.

In 2011, the amount of treated wastewater reached 270 million m^3. 98.29% of the treated water was carried out by advanced biological methods, and 1.22% of it by natural and 0.48% by biological treatment methods. In Turkey, approximately 500 million m^3 wastewater is treated by advanced biological treatment methods. Izmir is in the first place regarding wastewater treatment using advanced biological treatment as per European standards in Turkey.

In natural wastewater treatment facilities, the polluted water firstly comes to a compensating pool; secondly, it passes through

Table 5
Wastewater treatment facilities in Izmir [20].

Facility name	County name	Equivalent population/person	Capacity (m^3/day)	Treatment method
Cigli	Cigli	3,000,000	605,000	Advanced biological
Guneybati	Narlidere	100,000	21,600	Advanced biological
Havza	Menderes	100,000	21,600	Advanced biological
Bagarası	Foca	10,500	2100	Activated sludge
Halilbeyli	Kemalpasa	5500	1300	Activated sludge
Kozbeyli	Foca	2100	500	Activated sludge
Balikliova	Urla	5000	1000	Natural treatment
Foca	Foca	57,000	9763	Advanced biological
Gumuldur	Menderes	4000	1800	Activated sludge
Haciomerli	Aliaga	1250	250	Biodisc
IYTE (Izmir Institute of Tech.)	Urla	22,500	2250	Activated sludge
Selcuk	Selcuk	50,000	10,200	Natural treatment
Urkmez	Seferihisar	10,000	2000	Natural treatment
Urla	Urla	100,000	21,600	Advanced biological
Bayındır	Bayındır	40,000	6912	Advanced biological
Ayrancilar/Yazibasi	Torbali	40,000	6912	Advanced biological
Godence	Seferihisar	1250	250	Activated sludge
Torbalı	Torbalı	100,000	21,600	Advanced biological
Menemen	Menemen	100,000	21,600	Advanced biological
Seferihisar	Seferihisar	50,000	10,800	Advanced biological
Kemalpasa	Kemalpasa	70,000	12,960	Advanced biological
Aliaga	Aliaga	100,000	21,600	Advanced biological
Cakirbeyli	Torbali	1000	200	Natural treatment
Korucuk	Torbali	1000	200	Natural treatment
TOTAL			**803,997**	

an aerofilter (keeps out the stones and other materials) and finally it comes to stabilization pools. Only the Selcuk wastewater treatment facility uses aquatic plants. Mostly water hyacinth is used and other floating plants are allowed in the pools. First of all, wastewater is percolated by 5 cm scattered coarse grid and then it arrives at facultative pools. In this pool, suspended solids sink to the bottom and become stabilized. Other organic suspended materials, floating in the water, are absorbed by the plants in the pool. The water passes through a maturation pond and is kept there for 5 days. After all of these processes, the water is discharged into Kucuk Menderes River by drainage lines. Water hyacinth and other aquatic plants are harvested and are not used for any other processes [20,21].

5. Floating and aquatic plants

There are numerous technological methods to treat domestic sewage and industrial wastewater. On the other hand, natural treatment of wastewater became popular in the last few years. Especially in tropical–subtropical conditions, floating and aquatic plants are more preferred for natural treatment. The most known, important and still current plants are water hyacinth, duckweed and reeds [22]. These plants should have the optimum conditions in order to get high efficiency. The efficiency of the removal activity depends on the type of plant, working conditions, quality of feeding water and the required water [23].

Photosynthetic parts of floating plants are leaves that float on the water surface and the roots that grow to the bottom of water. These roots provide perfect filtration/adsorption and a medium to cultivate the bacteria. Nutrients help the roots to grow down, so the depth and the quality of the wastewater, the pretreatment of the water, the temperature and harvest are important parameters for floating plants. These plants hinder the ingress of sun beams through the water, which limits gas transfer between air and water. Hence, floating plants prevent the anaerobic conditions and inhibit algae production according to Biochemical Oxygen Demand (BOD), loading rate, hydraulic retention time and species of the plants [24].

Fig. 5. Water hyacinth planted in Ege University Solar Energy Institute.

5.1. Water hyacinth (Eichhornia crassipes)

Water hyacinth (*Eichhornia crassipes*) (Fig. 5), which is a widely prevalent aquatic weed in tropical/subtropical conditions, constitutes a potential biomass resource for different uses. Its high content of hemicellulose (30–55% of dry weight) can provide hemicellulosic sugars for bioconversion to fuel ethanol. Table 6 shows the compositions of some lignocellulosic biomaterials including water hyacinth. Under proper climatic conditions, water hyacinth is fast-growing plant and 0.26 ton of dry biomass is gained per hectare every season [25]. Under certain circumstances, the rate of proliferation is extremely rapid and it can spread over to large areas of water causing some problems. It grows in mats of

Table 6
Composition of various types of lignocellulosic biomass materials (% dry weight) [25,27].

Material	Cellulose	Hemicelluloses	Lignin
Algae (green)	20–40	20–50	–
Grasses	25–40	25–50	10–30
Hardwoods	45 ± 2	30 ± 5	20 ± 4
Hardwood barks	22–40	20–38	30–55
Softwood	42 ± 2	27 ± 2	28 ± 3
Softwood barks	18–38	15–33	30–60
Cornstalks	39–47	26–31	3–5
Wheat straw	37–41	27–32	13–15
Newspapers	40–55	25–40	–
Water hyacinth	15–25	30–55	3–4

up to 2 m thickness, which can reduce light and oxygen, change the water chemistry, affect the flora and fauna and cause significant increase in water loss due to evapotranspiration. It also causes practical problems for marine transportation, fishing and at intakes for hydropower and irrigation schemes. It is now considered a serious threat to biodiversity [26]. Despite these issues, water hyacinth has become a useful plant for the energy sector as a biomaterial and pretreatment for wastewater.

Water hyacinth can grow only in tropical and sub-tropical conditions. This plant covers the surface of water and decelerates the wind speed, which affects the temperature of water. Therefore, heat waves are stabilized. Besides, while water hyacinth's leaves has oxygen ability, its roots are almost anaerobic. That helps to balance of oxygen (air) and water ratio for the plant.

Water hyacinth is a popular plant for wastewater treatment because of its high rate of proliferation. It is used for both secondary treatment (BOD removal) and tertiary treatment (nitrogen, phosphorus, etc. nutrient removal). The retention time can change depending on the quality of the wastewater delivery, but generally it is 5–15 days.

The growth rate of water hyacinth is 220 kg/ha/day. Nitrogen removal rate is approximately 19 kg/ha/day if the amount of nitrogen is between 9 and 42 kg/ha/day. But this plant is easily affected by cold, and under 10 °C, the growth rate decreases.

5.2. Duckweed (Lemna minor)

Duckweed (*Lemna minor*) (Fig. 6) is a small floating aquatic plant within the family *Lemnaceae*. It proliferates through vegetative budding of new fronds and produces biomass more quickly than most other plants [28]. It has rapid proliferation, tolerance to high nutrient levels and low temperature, a wide range of pH tolerance and excellent nutrient uptake ability, because duckweed has been studied for nutrient recovery from wastewaters and as a biomaterial [29,30]. Duckweed's raw cellulose content is 14% [31] and it contains 35% crude protein [32]. These contents are available for microorganisms to generate and activate themselves to produce ethanol.

Duckweed is a freshwater plant and its size ranges between 1 and 3 mm with leaves. This plant is one of the smallest, easiest and fastest-growing plants. A cytokinesis creates a leaf and every leaf has the ability to generate 10–20 more new leaves as they are alive. Duckweed, growing in wastewater, duplicates its leaf number at 27 °C in 4 days. Most of the duckweed species produce a special substance named "turion", which keeps the duckweed alive at low temperatures [24].

Compared with most of green plants, the leaves of duckweed have less fiber because they don't need to prop up any leaves or its body. So, almost all tissues are metabolically activated and they may be used as feeding materials [33]. Duckweed metabolizes the decomposed organic materials and minerals in wastewater. This plant contains twice the amount of protein, lipid and nitrogen than water hyacinth and it contains 95% water, like water hyacinth.

Fig. 6. Duckweed – with water hyacinth – planted in Ege University Solar Energy Institute.

Chen et al. [34] used duckweed (L. punctate; strain of duckweed) as a novel feedstock for bioethanol production by *Saccharomyces cerevisiae*. The maximum glucose yield was 218.64 ± 3.10 mg/g dry matter, which is a 142% increase compared to the untreated mash. As a result of the fermentation experiments, 30.8 ± 0.8 g/l of ethanol concentration is obtained, and 90.04% of fermentation efficiency and 2.20 g/l(/)h of productivity rates were achieved [34].

Takagi et al. [35] chose water hyacinth as the raw material for bioethanol production. They also used *Saccharomyces cerevisiae* for fermentation and saccharified the water hyacinth with sulfuric acid and then treated it with cellulose. The original saccharified solution yielded glucose at 5.3 ± 0.2 g/l and reducing sugars at 9.7 ± 0.1 g/l. Concentration of the saccharified solution under vacuum at 70 °C yielded glucose at 21.5 ± 2.9 g/l and reducing sugars at 33.3 ± 2.1 g/l. 9.6 ± 1.1 g/l bioethanol was obtained from the concentrated saccharified solution at the end of the experiment [35].

Duckweed and water hyacinth are both glucose-sourced plants and their strains could be varied. According to glucose contents, bioethanol yields changes, and yields changing depends on cellulose amount and lignin shortage in the plant.

6. General process of second-generation bioethanol production

According to the projection of cellulosic ethanol as a feasible alternative to both petroleum and first-generation bioethanol, the significance of second-generation bioethanol production has increased. Selection of feedstock is easier than the first bioethanol production process since biomaterials are only lignocellulosic and they have no nutritional value [36].

The production process is the same as standard bioethanol production. The pretreatment is only different from the first-generation one. Lignocellulosic materials are difficult to hydrolyze due to carbohydrates obtained from cellulose; hence, there are mainly 5 steps to produce ethanol from lignocellulosic materials (Fig. 7): (i) pretreatment, (ii) cultivation and activation of microorganisms, (iii) fermentation, (iv) distillation and (v) dehydration [4].

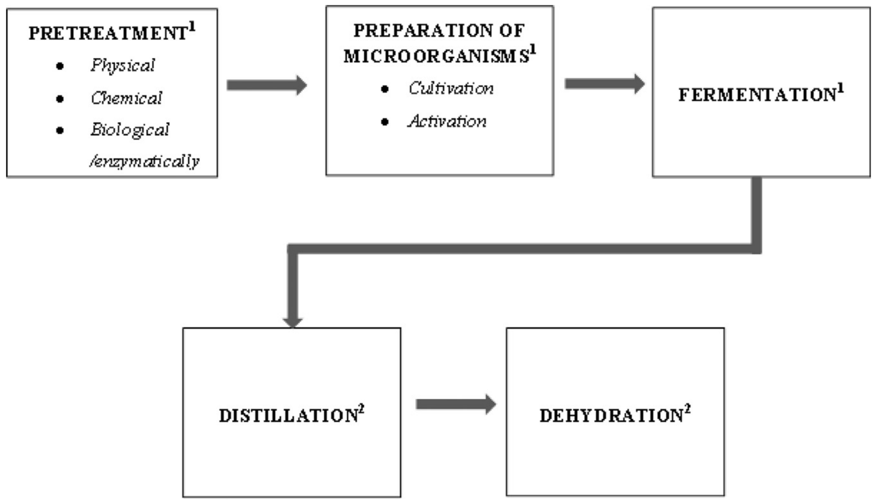

Fig. 7. Schematic process of second-generation bioethanol production ([1], [2] These steps can be run together).

Pretreatment is the most difficult part of the process; otherwise, fermentation is the main step of production. These two steps should be well planned and the risks that can occur during the liquefaction–saccharification steps, causing contaminations in the reactor, should be reduced.

Pretreatment methods for lignocellulosic materials are physical, chemical and biological/enzymatically. First of all, physical pretreatment should be used to increase the surface area followed by dilute or concentrated acid or alkali treatment step and, finally, enzymes and/or microorganisms. The second step, the chemical treatment step, can be skipped if the microorganism has an ability to disrupt the lignocellulosic structure. There are a few microorganisms that have a natural ability to disrupt the cellulose, such as *Pichia stipitis*, *Clostidium thermocellum* and some microorganisms that live in symbiotic or in mutualistic association with the host body such as *Ruminococcus flavefaciens* and *Prevotella bryantii* [37,38]. Selection of one of these organisms would be helpful in reducing energy consumption and also in saving time. Pretreatment, cultivation and activation of organism and fermentation would be carried out simultaneously because of the metabolism of microorganism.

6.1. Pretreatment methods

Lignocelluloses mainly consist of cellulose, hemicellulose and lignin; these components constitute approximately 90% of the dry matter in lignocelluloses, and the rest is composed of extract and ash. The basic structure of all woody biomass consists of three basic polymers: cellulose $(C_6H_{10}O_5)_x$, hemicellulose such as xylan $(C_5H_8O_4)_m$, and lignin $[C_9H_{10}O_3(OCH_3)_{0.9-1.7}]_n$ in trunk, foliage and bark. Cellulose and hemicellulose, which typically constitute two-thirds of the dry matter of the cell wall, are polysaccharides that can be hydrolyzed to sugars and then fermented to bioethanol. Process performance, i.e. bioethanol yield from biomass, is directly related to cellulose, hemicellulose and sugar concentration in the feedstock. The lignin cannot be used for bioethanol production [27,25].

Chemical composition of lignocellulosic materials is the main factor that affects the yield of bioethanol production. The structural and chemical compositions of lignocellulosic materials are extremely variable because of genetic and environmental influences and their interactions [39]. A typical chemical composition of lignocellulosic materials is 48 wt% C, 6 wt% H, 45 wt% O and the inorganic material, which is the minor component [40].

Pretreatment methods of lignocellulosic biomass are physical, chemical and enzymatic/microbiological. Physical methods increase the surface area of the biomaterials by mashing, cutting, graining with mill, etc. The chemical one is an intermediate process with acid or alkali solutions. This method helps hydrolyze the biomass and obtain carbohydrates. Lastly, the enzymatic/microbiological method is the most popular worldwide. Enzymes, such as cellulase, and microorganisms, such as *S. cerevisiae, P. stipitis* and *C. thermocellum*, have the special ability to convert cellulose and hemicellulose to polysaccharides, which are used by microorganisms to generate and produce bioethanol [41–43]. Gathering the 3 steps of the ethanol production process, pretreatment-microorganism cultivation and activation-fermentation; results in time and energy saving.

Gunnarsson and Petersen [44] estimated the presence of lignin to be about less than 10% in aquatic plants like water hyacinth and duckweed [44]. This value shows that they can be processed into biofuels efficiently. But the major problem of cellulosic bioethanol production is its high cost. The first part of the cost in biofuel production is the expense for the production of cellulase enzymes in bioreactors. The second part of the cost is pretreating lignocellulosic matter for its decomposition to intermediate components and the last part is the subsequent removal of the lignin [45].

Physical pretreatment methods of both water hyacinth and duckweed consist of drying, boiling, steaming, sonication, grilling and/or a combination of these methods. Harun et al. [46] compared the physical pretreatment methods. They used boiling, grilling, ultrasonication, drying and steaming. They analyzed the obtained hydrolyzed product with HPLC to estimate the reducing sugar content. The results showed that the combination of some methods is better than using only one method. For example, after drying in an oven, if water hyacinth is boiled, steamed and then ultrasonicated, the total sugar yield can rise up to 132.96 mg sugar/g dry matter. However, the study also showed that total sugar yield of only oven-dried and powdered water hyacinth is 155.13 mg sugar/g dry matter. Also, they found that the steamed samples of water hyacinth had a higher yield of sugar than the boiled samples [46].

Water hyacinth and duckweed display different types of polysaccharides. These sugars are mostly xylose, arabinose and glucose. The overall fermentable sugar could be available by acid hydrolysis up to 90% of the theoretical value of sugar present. Dilute acid process has to be conducted under 120–200 °C and between 103 kPa and 517 kPa. This process takes a little longer time than the concentrated acid hydrolyzing method. Sulfuric acid

is commonly used. When the acid is used, toxic compounds are formed such as furfural, acetic acid, formic acid, etc. and these acids inhibit fermentation and ethanol production [47].

The other way for pretreatment is alkali treatment using NaOH. Dilute NaOH (0.5%) treatment of water hyacinth causes swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in the crystalline structure, separation of lignin and carbohydrates and disruption of the lignin structure. Ammonia is also used to disrupt the lignin. Using these methods, delignification of water hyacinth is about 50–70% [47].

Pretreatment method could be considered as the most expensive step in the process. Enzymes, especially pure ones, are expensive and the chemicals are expensive because of the amount of usage. The costs of this step could be reduced by combining the pretreatment and microorganism activation–cultivation, which is the next step in the process.

6.2. Microorganisms

In the ethanol production process, the best microorganism should be chosen after the determination of the process type and equipment. The common characteristics of the microorganism should be as follows:

- High product yield per assimilation of substrate
- Capability of high fermentation
- Saturated ethanol tolerance
- Tolerance for temperature changes
- Ability to remain stable under fermentation conditions
- Tolerance for low pH values

Organisms should be in accordance with the medium owing to the biomaterials contents, process pathway and products. Bacteria and yeasts have some advantages and disadvantages in industrial ethanol production according to their abilities.

Ethanol production by yeast has a very large application in industrial production. The most used yeasts are *Saccharomyces cerevisiae*, *Pichia stipitis*, *Saccharomyces uvarum* (*carlsbergensis*), *Schizosaccharomyces pombe* and *Kluyveromyces* species. Yeasts can utilize various carbohydrates. Optimum pH value is between 3.5 and 6.0 and the temperature range is between 28 °C and 35 °C for ethanol production. Although the initial ethanol yield in thermophilic production processes is higher than in mesophilic production processes, ethanol production rate mostly decreases because of product inhibition [48].

Ethanol production is carried out with yeasts under anaerobic conditions by the Embden–Meyerhof–Parnas metabolic pathway. 2 mol of ethanol, carbon dioxide and ATP are produced from 1 mol of glucose. Theoretically, 0.51 g ethanol is obtained from 1 g glucose, but the actual yield cannot be 90–95% of the theoretical yield. However, some nutrients (e.g. carbon, nitrogen) are used to regenerate new cells and protect the existing cells [49].

Clostridium spp., *Escherichia coli, Klebsiella oxytoca* and *Zymomonas mobilis* are the most useful bacteria for production of bioethanol. *Zymomonas mobilis*, one of the bacteria used in bioethanol processes, uses only glucose or fructose. However, the other formed sugars also can be used, especially arabinose and xylose are the most desired sugars [50].

Several bacteria used in ethanol production produce different types of alcohols (e.g. butanol, isoprapyl alcohol, 2,3 – butanediol), including ethanol, in addition to organic acids (e.g. acetic acid, formic acid, lactic acid), poliols, kotones or some gases [51].

Roehr's study about the differences of ethanol production between bacteria and yeasts showed that bacteria are better than yeast because of their maximum production values. *Z. mobilis* can produce 120 g/l h per 100 g/l glucose. On the other hand, *S. cerevisiae* can produce only 29 g/l h while the same initial substrate amount is used [49]. All of the qualifications should be taken into consideration and the most useful microorganism should be chosen.

Lignocellulosic biomass is the most abundant renewable biological resource and the human food chain is also an attractive and relatively inexpensive raw material. Lignocellulosics are the second important resource for bioethanol production but it is the most important one because it can be easily harvested, can be used in bioethanol production and it is not a significant human food.

Most lignocellulosic raw materials have complex sugars. To reveal these sugars for microorganism's use, hydrolysis is required by chemical or enzymatic processes. This step is the most difficult and problematic step owing to the difficult degradability of cellulose. Cellulose has the strength structure to disrupt all sugar chains [52]. There are some microorganisms that can directly use cellulose to regenerate and also produce bioethanol. These microorganisms could directly be added to the fermentation medium, which contains cellulose without extra hydrolysis. They can save time and energy, thereby reducing the cost of the process. For these reasons, choosing the proper microorganism to produce bioethanol from lignocellulosic biomaterials is the key step. For the chosen biomaterial, the specific microorganism should be used to increase the yield and decrease the mean processing time.

Besides, microorganisms are mostly expensive as a strain and sometimes preparation of the conditions for cultivation could be costly. As mentioned in Section 6.1., combining the pretreatment and incubation processes, by choosing the correct microorganism, could reduce the total cost of the process. The correct microorganism could decompose the lignocellulose, use the own-produced carbon source and produce ethanol by fermentation.

6.2.1. Saccharomyces cerevisiae

Saccharomyces cerevisiae is a species of yeast that is a microorganism responsible for the most common type of fermentation. It is one of the most useful yeasts in the world and has been used for baking and brewing since centuries. *S. cerevisiae* cells are 5–10 μm in diameter and they can be reproduced by a division process known as budding (Fig. 8) [4].

S. cerevisiae is widely used in industrial ethanol production because of its ability to produce high concentrations of ethanol from hexoses and its high tolerance to ethanol and other inhibitory compounds. However, it is naturally unable to metabolize pentose sugars such as xylose and arabinose. Xylose is the second major fermentable sugar, which is present in hard woods and herbs. Because of this, its fermentation is essential for the economic conversion of lignocellulose to ethanol, which may provide an ideal alternative fuel source soon [53].

6.2.2. Clostridium thermocellum

Clostridium is a Gram-positive, anaerobic, fermentative bacterium that reproduces endospores. *Clostridium* are of different types: psychrophilic, mesophilic and thermophilic, and mostly causes food poisoning (Fig. 9).

Also, they are used to indicate the heat-time duration to make sterile canned foods. Thermophilic kinds of *Clostridias*, which are fermentative anaerobes, grow optimally at 60–65 °C and they have been of interest as potential producers of ethanol [55,56].

They use some kind of enzymes to degrade the cellulose. These enzymes are endo-b-glucanases, exoglucanases, cellobiose phosphorylases, cellodextrin phosphorylases and b-glucosidases [57]. These enzymes are often found in the cell structure called cellulosome. Multi-enzyme cellulose-degrading complexes are located and embedded on the external surface of the cell membrane.

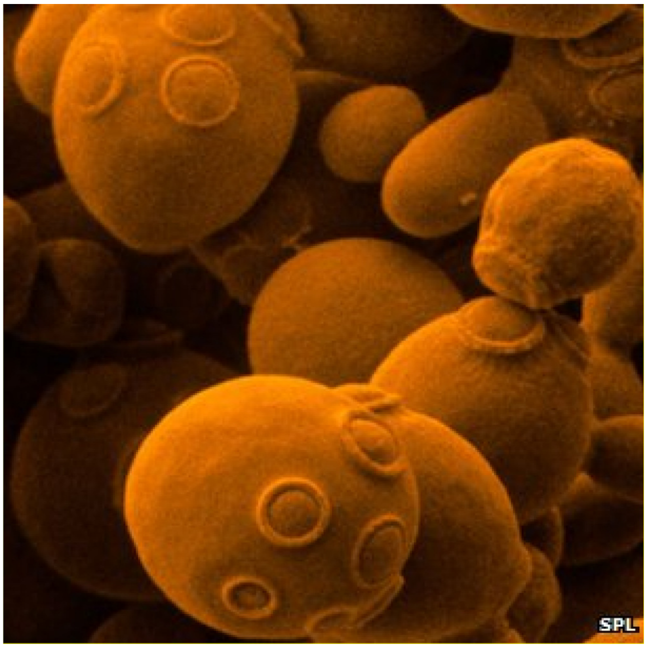

Fig. 8. *Saccharomyces cerevisiae* by colored electron microscope [54].

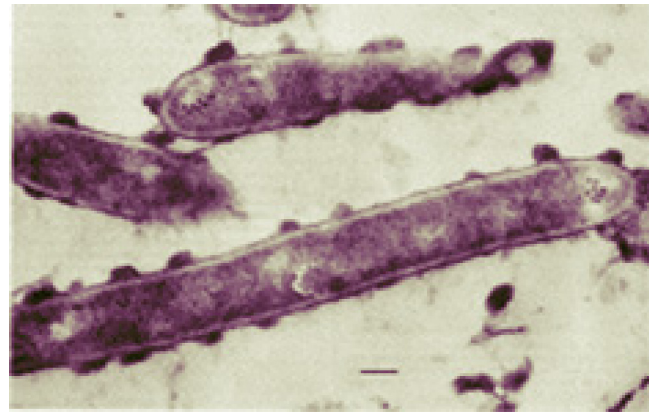

Fig. 9. *Clostridium thermocellum* by colored electron microscope [59].

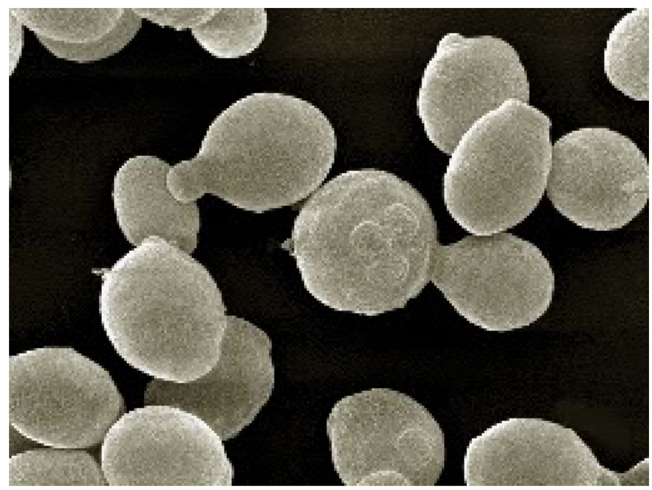

Fig. 10. *Pichia stipitis* by electron microscope [62].

A wide substrate range is a distinct advantage for any second-generation bioethanol process. As a result of experimental researches about bioethanol production, the process has focused on improving the substrate profiles of thermophilic *Clostridia*. For example, *C. thermocellum* ATCC 27405 and a strain of *C. thermohydrosulfuricum* have been reported to be able to ferment cellulose, cellobiose and a range of other carbohydrates. However, the extent of substrate consumption and specificity are varied between different strains and culture media. *C. thermocellum* has an ability to ferment pentose sugars. It also has broad substrate specificity and can grow on cellulose, cellobiose and xylooligomers as well as after adaptation on glucose, fructose and xylose.

Thermophilic *clostridia*'s central carbon metabolism is similar to glycolysis and the conversion of pyruvate to acetyl coenzyme A (CoA). Fermentative end products are lactate, acetate and ethanol [58].

6.2.3. *Pichia stipitis*

During bioethanol production, the microorganism must be able to ferment all monosaccharides present and also should withstand potential inhibitors in the hydrolysates (Fig. 10).

As mentioned, *Saccharomyces cerevisiae* is the most commonly used microorganism for ethanol production. It cannot ferment pentoses, which may form 45% of the raw material.

Pichia stipitis, which is the xylose-fermenting yeast, has shown promise for industrial applications because it ferments xylose rapidly with a high ethanol yield and apparently produces no xylitol [60]. *Pichia stipitis* is a mesophilic yeast and its pH range is between 5.0 and 6.5. Water hyacinth's hydrolysate mostly contains D-Xylose (12.4%/weight) and after pretreatment process, D-Xylose is liberated in the medium of fermentation, where microorganisms are added [61]. To consume less energy to heat the system and to obtain the highest yield from the process, the right microorganism should be chosen. *Pichia stipitis* has a better value from *Clostridium thermocellum* and *Saccharomyces cerevisiae* due to the ability of D-Xylose and better ethanol production yield than the other two microorganisms.

6.3. Fermentation

Under anaerobic conditions, microorganisms use the substrate gained from lignocellulosic biomaterials such as water hyacinth and duckweed. After this metabolic transformation, bioethanol is produced. Fermentation occurs by the Embden–Meyerhof–Parnas (EMP) process and glycolytic pathway's enzymes (Fig. 10).

If yeasts are used in ethanol production, the process is called zymosis. Zymosis is a process that reveals ATP in anaerobic metabolic pathways and it produces ethanol or milk acid, which is stored or released during the process. A zymosis metabolism could be occurring in plant or animal tissues. But mostly it is observed in microorganisms. Microorganisms use the ATP produced by zymosis for growing up and for cell reproduction.

In ethanol production, the zymosis' substrate is glucose. ATP is gained from substrate chain phosphorylation in the zymosis process. Reducer zymosis reaction creates ethanol and some other products [63].

In the ethanol production bioprocess, anaerobic destruction of carbohydrate follows the same process as glycolysis. However, pyruvate is decarboxylated into acetaldehyde by pyruvate decarboxylase. The decarboxylation product acetaldehyde cannot transfer to the lipoamide group. Finally, acetaldehyde is reduced to ethanol by both NADH and alcohol dehydrogenase [64].

6.4. Distillation and dehydration

Ethanol is obtained from the process medium before the last step in the distillation process. Ethanol can easily mix with water when it gets together at suitable conditions. Hence after the production process, ethanol is mixed with water (azeotropic

mixture) and other medium components. Distillation is supposed to take away the ethanol from the mixture. Ethanol's boiling point is 78.8 °C; depending on this value, the distillation column is used to take out from the top of the column about 95% ethanol with the remaining water (5%). But this percentage is not useful to utilize the ethanol in vehicle engines. So using with the water retaining structured column, it is easy to obtain 99.9% water-free ethanol, which could be used as transportation fuel in vehicles [4].

7. Conclusion

Turkey does not have enough petroleum reserves to be used as transportation fuel-oil. There are some areas that contain petroleum in the Southeastern Anatolian Region, but drilling the oil is an expensive process and the geographic conditions necessitate advanced technology equipment. Hence, Turkey imports nearly all of its petroleum and its expenditure is really high. Turkey is one of the countries that use petroleum with the highest price.

In additional, fossil fuels like petroleum are harmful for the environment. Gas emissions cause global warming and pose a threat to the next generation. For this reason, alternative energies, i.e. new and renewable energy resources, have become important.

Biomass energy could be considered as one the cheapest energy types in the world. The raw materials can be obtained using simple, basic systems. Bioethanol is becoming more popular than gasoline because bioethanol is a clean biofuel and can be used safely in transportation vehicles or in some other engines. Raw materials to produce bioethanol had been a problem, but second-generation bioethanol production has solved this problem.

Izmır's coordinates are suitable for agricultural activities and also allow harvesting plants more efficiently. If lignocellulosic biomaterials are taken into account, Izmir could be a self-sufficient province. Its air condition is suitable to cultivate water hyacinth and duckweed. Wetlands cover Izmir, and their qualities are suitable for the growth of water hyacinth and duckweed.

Wastewater treatment facilities in Izmir mostly use advanced biological treatment methods. These techniques are expensive and require more energy. However, natural wastewater treatment is easier and cheaper. Nature cleans up itself. There are no extra energy needs, only plants are needed in the ponds and pools. Hence water hyacinth and duckweed can be said to be the best aquatic plants to clean wastewater. They have the ability to remove organic pollutants such as nitrogen and inorganic pollutants such as industrial iron and other heavy metals.

After natural treatment, the cleansed water is discharged by the drainage lines and a cycle starts. This cycle helps to keep the environment sustainable. In Izmir, only five wastewater treatment facilities use the natural treatment method and just one of those facilities use aquatic plants for natural treatment. There is a rapid growth ability of water hyacinth and duckweed, especially in this area, which is suitable for harvesting them regularly, but these plants are not used for any processes after harvest.

Water hyacinth's cellulose and hemi-celluloses amounts are approximately 46.7 wt%. The total amount of C6 sugars in that amount are 26.3 wt% and consists of glucose (19.8 wt%) and galactose (6.5 wt%). Also, detectable C5 sugars (xylose and arabinose) in the water hyacinth leaves are in amounts of 11.5 and 9.0 wt%, respectively [65]. C5 and C6 sugars can be used to produce bioethanol in yeast or bacteria fermentation process. According to Anamed's research [66] water hyacinth's one hectare yield is between 20 and 120 ton of dry water hyacinth per year. If the average amount can be calculated, it is about 70 ton of dry matter of water hyacinth. So, if all the wetlands in Izmir are used for water hyacinth growth, 2.9 million ton/year (dry matter) can be harvested. So, approximately 757,000 ton/year C6 sugar and 594,000 ton/year C5 sugar can be used for bioethanol production. 1 g glucose (and almost all sugars) is converted into 0.51 g bioethanol by microorganisms [4]. Hence, 1.35 million tons of sugar can be used to produce 690,000 l/year of bioethanol in Izmir.

Duckweeds are very small plants but after the lag phase, the duckweed grew at a linear rate with time with a growth rate of approximately 30 g/m^2/d (dry weight); they consist of a good level of starch. If it is compared, duckweed can produce starch at a rate of approximately 28/ton/ha/year, where corn starch production is about 5 ton/ha/year. Hence, 509 mg reducing sugars is found per gram of dry duckweed. Fermentation of this solution using yeast gave an ethanol yield of 258 mg/g of the dry duckweed biomass [67].

Izmir has enough capacity, knowledge is getting better and technology is being researched and developed. Energy demand is increasing because of industrial development, and native energy sources can prove to be the best solution to save the future. Potential production amounts are adequate for second-generation bioethanol. Also, if high prices of the fuel-oil are taken into account, bioethanol shall be the most important fuel source for the world.

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